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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Our Ref: 1038-729 MIS:as

In re patent application

No. 08/931,721

Applicant: Barbara Papadopoulou et al

Title: MACROPHAGE-INFECTING PARASITES EXPRESSING A GRANULOCYTE MACROPHAGE COLONY STIMULATING FACTOR

Filed: September 16, 1997

Group No. 1645

Examiner: K. Masood

February 2, 2000

RESPONSE UNDER 37 CFR 1.116
EXPEDITED PROCEDURE

BY COURIER

The Commissioner of Patents
and Trademarks,
BOX AF,
Washington, D.C. 20231,
U.S.A.

Dear Sir:

This Communication is in response to the Office Action dated August 4, 1999.

Petition is hereby made under the provisions of 37 CFR 1.136(a) for an extension of three months of the period for response to the outstanding Office Action on this case. We enclose our cheque in the amount of the prescribed fees.

The Examiner rejected claims 1, 3 to 6, 10 and 21 under 35 USC 103(a) as being unpatentable over Moore et al in view of Wong et al. Reconsideration is requested having regard to the following discussion.

The Moore et al reference is concerned with a study of apoptosis of bone-marrow macrophages (or BMMs). As set forth in the introduction to the paper, in the absence of macrophage CSF, BMMs undergo a rapid decline in cell viability, resulting in the death of approximately 70% of the population within 48 hours and that BMMs infected with *L. donovani* demonstrate enhanced viability in the absence

of growth factor. As the authors note, these findings imply a soluble factor was secreted by *L. donovani* infected macrophages to the culture medium that may act in an autocrine manner to prevent cell death by apoptosis (see p. 2932, right-hand column, last para.)

The paper goes on to describe RT-PCR characterization of the cytokine expression profile of BMMs infected with *L. donovani.* This study found that the infection of BMMs by *L. donovani* promastigotes induced expression of four cytokine genes, namely GM-CSF, TNF-α, TGF-β and IL-6 (p. 2933, left-hand column). *L. donovani* infection of BMMs did not stimulate the expression of the M-CSF gene. Following the finding of expression of these cytokines by the BMMs, the authors studied whether any of the cytokine gene products could inhibit apoptosis of the BMMs and, to this end, BMMs (uninfected) were treated with recombinant versions of the cytokines. The authors found that rTNF-α and rGM-CSF were capable of inhibiting apoptosis, as demonstrated by a reduced level of fragmented DNA in the treated BMMs.

The next step in the study reported by Moore et al was to determine whether *L. donovani*-infected BMMs secreted TNF-α and GM-CSF into the culture medium. It was found that a significant level of TNF-α was present in the infected cell culture supernatants but no GM-CSF was detected (see p. 2933, right-hand column, last para.). Thus, while *L. donovani*-infected BMMs express the GM-CSF gene (as determined by the RT-PCR tests), the authors were unable to detect GM-CSF protein in supernatants from infected cells.

From these results, it was concluded by the authors that it is the secretion of TNF-α from the infected cells which is responsible, along with other factors, for inhibition of apoptosis of *L. donovani*-infected BMMs in the absence of M-CSF. The authors conclude their study with the following:

> "... two major observations are reported in the present study that we believe are related. The first is that *L. donovani* infection prevented apoptosis in BMMs and that this could also be mediated in part by LPG. Second, infected BMMs express a number of cytokine genes, which probably contributes to the prevention of apoptosis and may also be important in other aspects of the infection process. The enhancement of host cell viability could facilitate the spread of infection by increasing the number of host cells available for parasitization by *L. donovani* and by increasing the number of circulating infected macrophages for uptake by the sandfly vector."

Thus, the whole study presented by Moore et al relates to infection of BMMs by *L. donovani* and the effect of such infection on cell apoptosis of the BMMs. There is no hint or suggestion of any modification to the *L. donovani* itself to permit the *Leishmania* to express a foreign gene. The present invention, by contrast, provides a macrophage-infecting parasite, which is a strain of *Leishmania* and which is transformed by a plasmid containing a granulocyte macrophage colony stimulating factor (GM-CSF) and which parasite expresses GM-CSF.

It is submitted that the cited combination of prior art nowhere discloses or suggests the provision of any plasmid-transformed strain of *Leishmania* expressing a foreign gene, specifically GM-CSF.

It would seem, from the Examiner's comments, that the Examiner is regarding the BMMs infected by *L. donovani* as being a "macrophage infecting parasite", when the Examiner states:

> "... to transform Moore's *Leishmania donovani* "macrophage infecting parasite", which intrinsically also expresses the cytokines IL-6, TNF-α and TFGF-β ..." (emphasis added).

However, this cannot be a correct interpretation. Claim 1 requires the provision of a macrophage-infecting parasite which is a strain of *Leishmania*. Thus, the parasite is one which is a strain of *Leishmania* and is macrophage infecting. In Moore et al, the BMMs are infected by a parasite which is a strain of *Leishmania*. The *L. donovani* does not "intrinsically" express cytokines IL-6, TNF-α and TGF-β, but rather induces the BMMs to express such genes.

The Examiner goes on to indicate the transformation is:

> "... with the human and murine constructs of Wong et al ... in order to increase GM-CSF protein levels to detectable levels."

The Wong et al reference teaches the GM-CSF gene and its recombinant expression. Simply because there are no detectable levels of GM-CSF in the supernatants of BMMs infected with *L. donovani* provides no motivation for there to be such expression, since such is merely an observation of an experiment without conclusion as to its effect. BMMs infected by *L. donovani* are induced to express, from the BMMs, four cytokine genes, including GM-CSF, provides no motivation to transform a strain of *Leishmania* with a plasmid containing a GM-CSF gene so that

the strain of *Leishmania* itself expresses GM-CSF. There is no motivation whatsoever provided by the Moore et al teaching to achieve detectable levels of GM-CSF protein.

As to the assertion:

> "Additional motivation resides in that such transformation of *Leishmania donovani* with a GM-CSF expression plasmid would more efficiently prevent apoptosis of BMMs which, therefore, increase BMM survivability and viability (pg. 2935, last two paragraphs, and last two paragraphs, pg. 2935); and thereby decrease overall *Leishmania* infectivity and survivability..."

there is no such motivation provided. The cited portions of the paper merely state that cellular cytokine gene expression from the BMMs was induced by infection by *L. donovani* and that such cytokines include GM-CSF. As already noted, the authors conclude that the enhancement of host cell viability could facilitate the spread of infection rather than decrease overall *Leishmania* infectivity and survivability, as suggested by the Examiner.

Accordingly, it is submitted that the only macrophage-infecting parasite described by Moore et al is *L. donovani* and the paper describes the effect of infection of BMMs by this macrophage-infecting parasite on cell apoptosis. While there is an observation that such infection induces expression of cytokines, including GM-CSF, by the BMMs, but that GM-CSF is not detected in supernatants of the parasite-infected BMMs, there is no motivation whatsoever to transform the macrophage-infecting parasite with a plasmid containing the GM-CSF gene so as to permit expression of GM-CSF by the parasite.

Having regard to the above, it is submitted that claims 1, 3 to 6, and 10 to 21 are patentable over the applied art and hence the rejection thereof under 35 USC 103(a) as being unpatentable over Moore et al in view of Wong et al, should be withdrawn.

The Examiner also rejected claims 1, 3 to 8, 10 and 21 under 35 USC 103(a) as being unpatentable over Moore et al in view of Wong et al and further in view of Laban et al. Reconsideration is requested, having regard to the following discussion.

The relevance of the combination of the Moore et al and Wong et al references to the patentability of claims 1, 3 to 6, 10 and 21 has been discussed

above and requires no further elaboration. The Examiner has added claims 7 to 8 to the rejection and added the Laban et al reference. Claim 8 was deleted in response to the prior Office Action.

Claim 7 recites that the GM-CSF gene is expressed using the α-tubulin intergenic sequence of *Leishmania enrietti*. It is submitted that this disclosure provides no disclosure which would remedy the basic defect of the combination of Moore et al and Wong et al to transform a strain of *Leishmania* to achieve expression of GM-CSF from the *Leishmania* strain.

Accordingly, it is submitted that claims 1, 3 to 8, 10 and 21, to the extent they remain in the application, are patentable over the applied art and hence the rejection thereof under 35 USC 103(a) as being unpatentable over Moore et al in view of Wong et al and further in view of Laban et al, should be withdrawn.

The Examiner rejected claim 9 under 35 USC 103(a) as being unpatentable over Moore et al, in view of Matlashewski. Reconsideration is requested having regard to the following discussion.

Claim 9 is dependent on claim 1 and recites that at least one gene of the parasite contributing to its virulence has been functionally disabled. This modification is in addition to the modification to the *Leishmania* strain required by claim 1.

The Moore et al reference has been fully discussed above and such discussion need not be repeated here. The Examiner's discussion of this reference would not appear to suggest that there is any disclosure by Moore et al to transform a strain of *Leishmania* by a plasmid containing a GM-CSF gene to provide a transformed *Leishmania* strain which expresses GM-CSF.

Even if the rejection is interpreted to include the Wong et al reference, the combination of Moore et al with Wong et al and the defects of such combination have been fully discussed above and again do not need repeating. The Matlashewski reference refers to the generation of attenuated strains of *Leishmania* and, in no manner, remedies the defects of Moore et al, even if considered in conjunction with Wong et al.

Accordingly, it is submitted that claim 9 is patentable over the applied art and hence the rejection thereof under 35 USC 103(a) as being unpatentable over Moore et al in view of Matlashewski, should be withdrawn.

It is believed that this application is now in condition for allowance and early and favourable consideration and allowance are respectfully solicited.

Respectfully submitted,

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